SOME EXPERIMENTS ON THE ORDER OF SUCCESSION OF THE SOMITES IN THE CHICK

PROFESSOR MARIAN E. HUBBARD

WELLESLEY COLLEGE

THE experiments described in the present paper were performed at the University of Chicago during the year 1903–04, under the direction of Professor F. R. Lillie, to whom thanks are due for much advice and suggestion. In the course of the preparation of the data for publication during the last summer I learned that another investigator, Mr. J. Thos. Patterson ('07), had hit upon the same problem, and his results appeared before this article could be published. It has been suggested, however, that the work described may be of value in confirming Mr. Patterson's conclusions.

The problem, suggested by Professor Lillie, was the investigation of the statement, so generally made by embryologists, that, in the chick, somites arise in front of the one which is formed first. An examination of the most important of these statements will make clearer the nature of the problem. The estimates of von Baer ('28) and His ('68) did not require serious consideration, for they were not based upon a close study of this point. That of Kupffer and Benecke ('79), who thought that three or four somites arose in front of the one which first appeared, was founded upon an examination of a rather wide series of embryos, but only in surface view. Miss Platt's ('89) work rested upon a study of sagittal sections, and as it was altogether a careful examination of the subject, my attention was directed particularly to her conclusions. Briefly, her account of the formation of the somites is as follows:[1] The first cleft divides two

[1] *Loc. cit.*, pp. 177, 178.

forming somites. The somite behind the cleft is called the first one in the series. The one anterior to it, protovertebra *a*, forms slowly, while four or five are making their appearance behind. After five or six somites are visible in all, another, protovertebra *b*, arises slowly in front of *a*. Protovertebra *b* is said to be rudimentary, never becoming completely cut off from the mesoderm in front.

It will be noted in this account that although two somites are described as arising in front of the one first formed, in reality there is but one to be considered—protovertebra *b*—for protovertebra *a* makes its appearance at the same time with the one behind it. An examination of Miss Platt's sections[2] would lead one to agree in the main with her account of the order of formation of the somites, except in regard to the appearance of protovertebra *b*, whose growth has to be followed in a series of sections from different embryos at successively older stages. The difficulty of identifying a growing somite in this way casts much doubt upon even its existence, and it was to test the question therefore that these experiments were devised.

The aim of the experiments was to mark or destroy, in embryos with a small number of somites (not more than five or six) the most anterior somite on one side, and so to determine whether any more were later formed in front of this. The ideal stage to have secured would have been that of an embryo with only a single pair of somites, but repeated failures to obtain this condition verified the statement made by Miss Platt,[3] founded upon a study of sections, that the first cleft occurs between two forming somites. An operation, then, even as early as at the time of the first cleft would have had to take into account the first two pairs of protovertebræ.

The methods employed in the experiments were in general similar to those used by Mr. Patterson. For open-

[2] *Loc. cit.*, Plate I.

[3] *Loc cit.*, p. 177.

ing and sealing the egg Miss Peebles'[4] method was followed. For destroying the somites two fine depilatory needles, ground to a hair point on an oil-stone, were used, one, at the negative electrode, touching the albumen, the other, at the positive electrode, serving to prick the somite which was to be marked or destroyed. For the current four Samson dry-battery cells, each with an electromotive force of 1.5 volts, were connected in series. To prevent infection the instruments were sterilized in a flame. With this method of disinfection, 15 out of 84 embryos, or 18 per cent., were lost, but as the loss was occasioned by the sticking of the blastoderm to the shell, it can not be stated that it was not due in part to causes other than bacterial infection. A Zeiss dissecting stand was used for the operations, with lenses magnifying six diameters, and whenever possible the work was done with the bright sunlight shining in upon the blastoderm. So great is the variation in distinctness of embryos at this early age, that even with the best of light the somites could not, except in a comparatively small number of cases, be counted with certainty. In the embryos, however, which were distinct, there was no room for doubt as to their exact condition at the time of the operation.

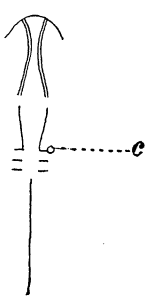

FIG. 1. Embryo 50. Sketch made at time of operation. c = place of operation.

From several experiments, the results of which furnish evidence for the solution of the problem, the following case has been selected for description:

Number 50 was operated upon after 30 hours of incubation. The operation was performed with the sun shining in upon the blastoderm, the embryo was distinct, and its three somites were readily counted. Fig. 1 is a sketch made at the time, showing the place of the operation, in which, it was noted, the needle passed obliquely inward.

[4] *Loc. cit.*, p. 406.

Fig. 2 shows the same embryo after nineteen more hours of incubation. The heart was beating when the egg was opened. The embryo was preserved in picrosulphuric-acetic acid, stained in Conklin's picro-hæmatoxylin, and mounted in xylol balsam. The drawing was made with the aid of the Abbe camera.

The first right somite is noticeably smaller than its fellow on the left, there is no break between it and the mesoderm in front, and only the posterior part of it shows the radial arrangement of cells which is characteristic of the normal somite. The scar of the operation shows at the side. A deeper examination in this region reveals, mediad of the scar, a clear area extending into the limits of both the first and the second somite of that side, indicating that the injury reached inward from the point of entrance of the needle. The second somite is also incomplete on its dorsal antero-lateral corner, as shown in the figure. Except for these injuries and the bend to the right which may have been caused by the operation, the embryo appears normal, the break in the neural tube at the anterior end being the result of pressure of the cover-glass.

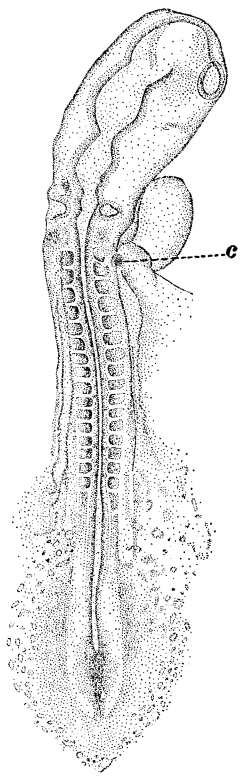

FIG. 2. Embryo 50, incubated 19 hours after operation. *c* = scar of operation. × 20.

Whatever else this experiment proved, it showed clearly that not more than two somites could arise in front of the one which is first formed. This of course shut out at once the hypothesis of Kupffer and Benecke, who assumed that three or four somites are probably formed in front.

Applying Miss Platt's description of the order of ap-

pearance of the somites to this case, it would seem that this embryo must have had the first of the two anterior somites, protovertebra *a*, already partly formed, at the time of the operation, and that there should, therefore, have been one more, protovertebra *b*, to arise in front of this. But no such somite appears in Fig. 2, and its absence led to the conclusion that there is no such somite as protovertebra *b*, in other words, that but one somite is formed in front of the first cleft which appears. The simplest explanation of Miss Platt's error is that she mistook protovertebra *a* in sections of older embryos for protovertebra *b*. This is much more probable than that she could have mistaken, as Mr. Patterson suggests,[5] the most posterior transitory shallow depression in the head mesoderm for the first cleft.

If it be objected that the experiment does not prove that one or two somites may not arise in front of the one first formed, it may be said that if they do arise, the rate of their formation, compared with the rate of formation of those that appear behind, is contrary to the description of this process by Miss Platt,[6] according to whom the rate of formation is much greater behind the first formed somite than it is in front. Either then somites are not formed in front, or, if they do arise, the description of the rate of their formation is not correct.

In conclusion, then, this experiment, in proving that not more than two somites could arise in front, showed the inaccuracy of Kupffer and Benecke's estimate of the number formed.

It showed further, in regard to Miss Platt's work, either that her description of the time of formation of the somites was incorrect, or, if development proceeds according to her account, that no somites, except the rudimentary one, arise in front of the first cleft.

Thus the result of the experiments, with reference to the condition of the problem up to the time when Mr.

[5] *Loc. cit.*, pp. 129, 132.

[6] *Loc. cit.*, p. 177.

Patterson began his work upon it, was to throw the burden of proof on those who claimed that somites do arise in front of the one first formed, rather than on those who held that, in their formation, they obey the laws of progressive differentiation which govern the early development of birds.

TEXT REFERENCES.

1828. Baer, Karl Ernst von. Entwickelungsgeschichte der Thiere.

1868. His, Wilhelm. Untersuchungen über die Erste Anlage des Wirbelthierleibes.

1879. Kupffer, C., und Benecke, B. Photogramme zur Ontogonie der Vögel. *Verh. der Ksl.-Leop.-Carol.-Dtsch. Akad. d. Naturf.*, Bd. 41, pp. 149–196.

1907. Patterson, J. Thos. The Order of Appearance of the Anterior Somites in the Chick. *Biol. Bull.*, XIII, pp. 121–133.

1898. Peebles, Florence. Some Experiments on the Primitive Streak of the Chick. *Arch. f. Entw.-Mech.*, Bd. VII, pp. 405–429.

1889. Platt, Julia B. Studies on the Primitive Axial Segmentation of the Chick. *Bull. Mus. Comp. Zool.*, Harvard, Vol. XVII, No. 4, pp. 171–179.